Patent Claims

1. The use of a labeled sphingosine for determining whether an activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway is present in a sample or not, or determining the extent of said activity.

2. A method for determining whether an activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway is present in a sample or not, or determining the extent of said activity comprising the steps of
   a. contacting living cells comprised in an appropriate culture medium with a labeled sphingosine for a predetermined period of time so that an enzymatic product can be formed,
   b. separating the enzymatic product formed in step a., and
   c. determining the amount of enzymatic product formed.

3. A method for determining whether an activity of a sphingosine kinase is present in a sample or not, or determining the extent of said activity comprising the steps of
   A. contacting a labeled unphosphorylated sphingosine with
      - a sample which sample optionally comprises a sphingosine kinase and
      - a phosphate source,
      for a predetermined period of time so that an enzymatic product can be formed,
   B. adding to the mixture of step A. an aqueous buffer solution and organic solvent which is able to form two phases in combination with water,
   C. separating the phases obtained in step B,
   D. determining the amount of enzymatic product in the aqueous phase obtained in step C..

4. A method for identifying an agent that modulates the activity of a sphingosine kinase comprising the steps of
   a. contacting a labeled unphsophorylated sphingosine with
      - a phosphate source, and
      - a sphingosine kinase
      for a predetermined period of time so that an enzymatic product can be formed,
   a1. in the absence of a candidate compound, and
   a2. in the presence of a candidate compound,

b. adding to the mixture of step a1 and of step a2 an aqueous buffer solution and organic solvent which is able to form two phases in combination with water,

c. separating the unreacted labeled sphingosine from the enzymatic product formed in steps a1. and a2., e.g. according to claim 1, steps b. and c.,

d. detecting the amount of enzymatic product obtained in step a1. and in step a2 and determining whether there is a difference in the amount of enzymatic products formed in step a1. and step a2.,

e. choosing an agent that modulates the activity of a sphingosine kinase as determined in step d.

5. A method for identifying an agent that modulates the activity of a phosphatase involved in the sphingolipid pathway comprising the steps of

A. contacting a labeled phosphorylated sphingosine with living cells comprised in an appropriate medium for a predetermined period of time so that an enzymatic product can be formed,

A1. in the absence of a candidate compound, and

A2. in the presence of a candidate compound,

B. separating the unreacted labeled phosphorylated sphingosine from the enzymatic product formed in steps A1. and A2.,

C. detecting the amount of enzymatic product obtained in step A1. and in step A2 and determining whether there is a difference in the amount of enzymatic products formed in step A1. and step A2.,

D. choosing an agent that modulates the activity of a phosphatase involved in the sphingolipid pathway as determined in step C.

6. A method for determining whether in a sample sphingosine kinase-1-activity or sphingosine kinase-2-activity or both or no sphingosine kinase activity is present comprising the steps of

α. contacting

α1. a labeled unphosphorylated sphingosine with a sample which sample optionally comprises sphingosine kinase-1-activity, or sphingosine kinase-2-activity, or both, or no sphingosine kinase activity, with a phosphate source,

α2. a labeled unphosphorylated sphingosine with a sample comprising a defined amount of sphingosine kinase-1-activity with a phosphate source,

α3. a labeled unphosphorylated sphingosine with a sample comprising a defined amount of sphingosine kinase-2-activity with a phosphate source for a predetermined period of time so that an enzymatic product can be formed,

β. separating the unreacted compound of a labeled sphingosine from the enzymatic product formed in steps α1., α2. and α3., e.g. according to method steps b. and c. as defined in claim 1, and

γ. determining and comparing the phosphate conversion rate in steps α1., α2. and α3.

7. A method for differentiating whether a test compound is capable to mediate the activity of a sphingosine kinase-1 and/or a sphingosine kinase-2 comprising the steps

i. contacting an unphosphorylated compound of formula I with a phosphate source and with

i1. a sphingosine kinase-1,

i2. a sphingosine kinase-2,

- in the absence of a test compound, and
- in the presence of a test compound

for a predetermined period of time so that an enzymatic product can be formed,

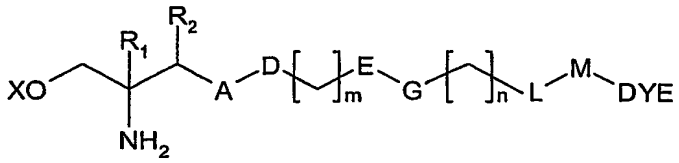
ii. separating the unreacted unphosphorylated compound of formula I from the enzymatic product formed in steps i1. and i2., e.g. according to method steps b. and c. as defined in claim 1, and

iii. determining and comparing the phosphate conversion rate in steps i1. and i2..

8. A kit for kit for determining the activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway comprising as a main component a labeled sphingosine and instructions for using said kit.

9. A kit of claim 8 for use in the identification of an agent that mediates the activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway.

10. The use, the method of any one of claims 1 to 7, or a kit of an one of claims 8 or 9 wherein the labeled sphingosine is a compound of formula



I

wherein

R_1 is H or (C_{1-4})alkyl,

R_2 is H, OH or oxo,

X is H or $(HO)_2PO$,

A–D, E–G and L–M independently of each other is a group

CH_2-CH_2, CH=CH, C≡C, CH_2-phenyl, phenyl-CH_2, CH_2-CH_2-phenyl, CH_2-NH, CH_2-N((C_{1-4})alkyl), NH-CH_2, N((C_{1-4})alkyl)-CH_2, O-CH_2, CH_2-O, phenyl-O, O-phenyl, CH_2-phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C_{1-4})alkyl), N(C_{1-4})alkyl)-CO, NH-SO_2, SO_2-NH, N((C_{1-4})alkyl)-SO_2,

or one group out of A–D, E–G and L–M is absent

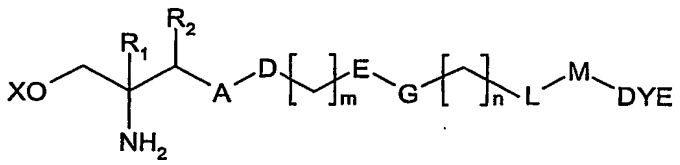
m is a number selected from 0 to 12,

n is a number selected from 0 to 12,

and m plus n is a number selected from 0 to 14,

the group DYE is a group selectively detectable in a compound of formula I by physical means, with the proviso that at least one of E-G and L-M is selected from the group consisting of CH_2-NH, CH_2-N((C_{1-4})alkyl), CH_2-O, phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C_{1-4})alkyl), N(C_{1-4})alkyl)-CO, NH-SO_2, N((C_{1-4})alkyl)-SO_2.

11. A compound of formula



I

wherein

R_1 is H or (C_{1-4})alkyl,

R_2 is H, OH or oxo, e.g. H or OH,

X is H or $(HO)_2PO$,

A–D, E–G and L–M independently of each other is a group

CH_2-CH_2, CH=CH, C≡C, CH_2-phenyl, phenyl-CH_2, CH_2-CH_2-phenyl, CH_2-NH, CH_2-N((C_{1-4})alkyl), NH-CH_2, N((C_{1-4})alkyl)-CH_2, O-CH_2, CH_2-O, phenyl-O, O-phenyl, CH_2-phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C_{1-4})alkyl), N(C_{1-4})alkyl)-CO, NH-SO_2, SO_2-NH, N((C_{1-4})alkyl)-SO_2,

or one group out of A–D, E–G and L–M is absent,

m is a number selected from 0 to 12,

n is a number selected from 0 to 12,

m plus n is a number selected from 0 to 14, and

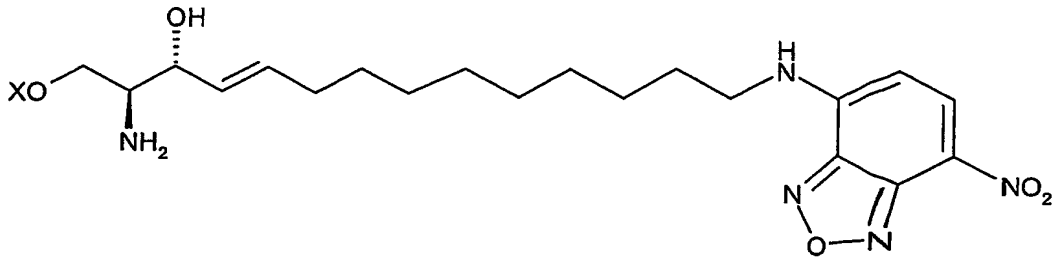
the group DYE is a group selectively detectable in a compound of formula I by physical means,

with the proviso that

- at least one of E-G and L-M is selected from the group consisting of

CH_2-NH, CH_2-N((C_{1-4})alkyl), CH_2-O, phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C_{1-4})alkyl), N((C_{1-4})alkyl)-CO, NH-SO_2, N((C_{1-4})alkyl)-SO_2, and

- a compound of formula



wherein X is as defined above, is excluded.

12. The use of a fluorescent labeled sphingosine of formula I as defined in claim 11 in a high-throughput assay, e.g. for the identification of an agent that modulates the activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway.

13. An agent which is capable to mediate an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway, which agent is identified by a method of any one of claims 4 or 5.

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